Vol. XXI. *September, 1911.* *No. 4.*

BIOLOGICAL BULLETIN

A STUDY OF THE RELATION OF TISSUE DIFFERENTIATION TO RATE OF GROWTH DURING REGENERATION.[1]

W. E. ALLEN.

ACKNOWLEDGMENTS.

This work was done under the direction of Dr. Chas. Zeleny, whose advice and criticism have been invaluable. I am also indebted to Professor H. B. Ward for suggestions and encouragement, to Mr. S. F. Prince for help with the drawings, to Mr. W. Scott, of Indiana University, for *Amblystoma* material efficiently supplied and to the Illinois State Laboratory of Natural History for the loan of valuable literature.

OBJECT.

The object of this study was to find whether the greatest speed of regeneration occurs before or after or coincident with the completion of tissue differentiation. If the same correlation holds that was noted by Minot (1908) in ordinary growth, we should expect to find the rate of growth decreasing after the major tissues have been well developed and still more so after differentiation is essentially complete. In the hope of securing definite information the present effort has been made to secure data in that definite line, using two distinct types for study.

EXPERIMENT I. A MATURE ANIMAL.

THE OLIOGOCHÆTE WORM, *Limnodrilus claparedianus*, Ratzel.

Material and Methods.

The work was begun in early October, 1910. At that time there was not much choice of material for such investigation as a

[1] Contributions from the Zoölogical Laboratory, University of Illinois, under the direction of Henry B. Ward, No. 9.

preliminary survey of the local field soon showed. Lack of better material was therefore the principal reason for choosing *Limnodrilus*. Of points distinctly in its favor, the most prominent were accessibility, hardiness, adaptability to laboratory conditions and rapidity of regeneration. On the other hand, the small size, irritability, quickness and sandy food caused some serious difficulties. The specimens used were taken from a sandy slough margin just north of Urbana Fair Grounds. All were in good condition and in a few hours appeared quite at home under laboratory conditions.

The effort was made to select 120 worms of equal median size, but their activity was so great and their appearance changed so quickly that the results showed considerable variation. Great care was taken to make exact transverse cuts at the middle, but here again, activity interfered and there was a considerable percentage of errors in cutting. For operation each worm was placed on a paraffine block and a quick even cut was made with a sharp, thin scalpel. Anterior parts only were retained. Those of the first five worms bisected were at once stupefied in weak chloretone solution and killed in Gilson's sublimate mixture (Lee, 1900). All other worms were handled in the same way. The second five was kept alive 1 hour after operation. All others were placed in 10 cm. Petri dishes in which was about 4 c.c. of sterilized native mud with 30 c.c. of tap water. Ten were placed in each dish except by miscount due to disappearance in the mud. Two dishes with unoperated worms were kept for check. One hundred were bisected. Water was changed in the Petri dishes twice daily immediately after taking the temperature of the water already in the dishes. The highest temperature recorded was 28° C., the lowest 21° C. The greatest fluctuation in 12 hours was 5°. While it was unfortunate that laboratory conditions were not such as to permit more uniform temperature, it is hardly probable that even this variation seriously affected these hardy worms, none of which died. Light and other conditions were kept as nearly uniform as possible. Direct sunlight was avoided altogether.

As noted before the first five was killed immediately after operation, the second in 1 hour, third in 6 hours, fourth in 12

TABLE I.

Limnodrilus.

	Individual Worm Number.	Length of Single Old Segment in mms.	Length of Whole Regenerated Part in mms.
1st day.....	7*a*	.418	.114
	7*b*	.432	.057
	7*c*	.425	.114
	7*d*	.432	.076
	7*e*	.342	.038
2d day.....	7*f*	.418	.083
	7*g*	.380	(.034)
	7*h*	.361	.285
	7*i*	.380	.380
	7*j*	.418	.190
3d day.....	8*a*	.342	.342
	9*a*	.494	.228
	9*b*	.304	.300
	10*a*	.380	(.114)
	10*b*	.418	.234
4th day.....	8*c*	.418	.608
	8*d*	.456	.380
	9*c*	.342	.608
	9*d*	.430	.684
	10*c*	.418	.284
	10*d*	.418	.608
5th day.....	8*e*	.418	1.254
	8*f*	.266	.988
	9*e*	.304	.722
	10*e*	.380	.950
	10*f*	.370	.912
6th day.....	8*g*	.456	1.824
	9*g*	.418	1.824
	9*h*	.418	2.012
	10*g*	.342	1.824
	[b]10*h*	.418	2.204
7th day.....	8*i*	.284	3.648
	8*j*	.456	3.800
	8*k*	.418	(1.634)
	9*i*	.322	2.546
	9*j*	.304	1.900
	10*i*	.418	(.988)
	10*j*	.380	2.888
	10*k*	.380	3.268
	10*l*	.494	4.180
	10*m*	.380	(.076)
8th day.....	11*a*	.456	3.800
	11*b*	.342	4.788
	12*a*	.456	4.066
	13*a*	.418	5.928
	14*a*	.456	5.016

Explanation of Table I.—Table I. is a copy of the records, in millimeters, from which the growth rate for the total regenerated part was calculated. Numbers omitted because of undue influence upon the average are placed in parenthesis.

TABLE I.—*Continued.*

Limnodrilus.

	Individual Worm Number.	Length of Single Old Segment in mms.	Length of Whole Regenerated Part in mms.
9th day.....	11*c*	.456	7.448
	11*d*	.456	5.016
	12*b*	.380	7.400
	13*b*	.494	8.132
	14*b*	.456	6.840
10th day.....	11*e*	.342	7.400
	12*c*	.456	7.068
	12*d*	.532	(5.928)
	13*c*	.418	7.980
	14*c*	.380	8.626
11th day.....	11*f*	.418	6.346
	12*e*	.456	9.196
	12*f*	.456	7.670
	13*d*	.494	10.754
	14*d*	.361	7.752
12th day.....	11*g*		9.1
	12*g*		9.4
	13*e*		9.
	13*f*		9.6
	14*e*		9.8
15th day.....	11*h*		12.7
	12*h*		10.9
	13*g*		13.3
	13*h*		11.5
	14*f*		18.9
18th day.....	11*i*		10.9
	12*i*		17.2
	13*i*		16.8
	14*g*		16.3
	14*h*		13.7

hours and fifth in 24 hours. After that a group of five was taken each day to the twelfth day, the remainder on the fifteenth and eighteenth days. Since the specimens had to be killed for tissue study it was necessary to calculate the rate of growth from the condition in successive groups after killing instead of using periodic measurements of living worms.

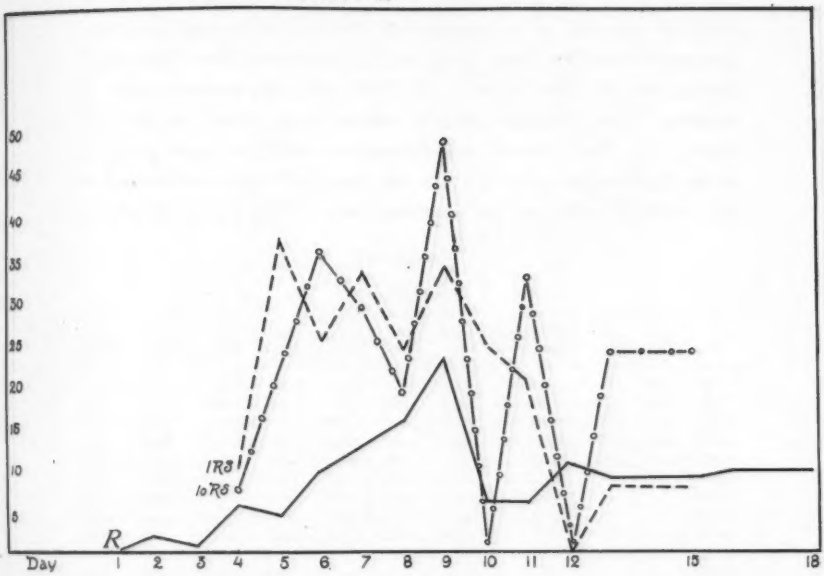
DATA.

The following sets of measurements were taken:

1. The total length of the regenerated part was measured by eyepiece micrometer under low power as the specimens lay in

cedar oil in a watch glass. (Table I. and Chart 1.) Crooked specimens caused some inaccuracy here.

Chart I.



Explanation of Chart I.—A graphic representation of the records contained in Tables I. and II. The continuous line *R* is for the total regenerated part. The broken lines (1 *RS* and 10 *RS*) are for the first and tenth regenerated segments respectively. Each 1/20 inch rise in the polygon represents an increase in rate of growth of 0.1 mm. for *R* and 0.001 for 1 *RS* and 10 *RS*.

2. At the same time and under the same conditions an old segment, about tenth from the level of cut, was measured in order to get a basis of comparison of general results. (Table I.)

3. Measurements were made after mounting on slides, some after sectioning but most in cedar oil. In this way some of the errors due to crookedness were detected though the general result is not essentially different. (Table II.)

4. Every tenth segment of the regenerated part was measured for length. (Table II.)

As these measurements form the only basis for the construction of a polygon (Chart I.) to graphically illustrate the rate of

growth it is worth while to give them a little general discussion. There are sources of error, which being well recognized, have been checked upon and guarded against as much as possible. (1) The measurements were made and the segments counted without the use of a mechanical stage. Since the specimens possessed very few good guide marks there may have been slight errors due to this cause. (2) Only one count was made in simpler cases although several counts were made in doubtful cases. (3) The correct measurements may be misleading at times because of variability in the speed of regeneration due to lack of uniformity in the bisecting cuts. This is due largely to

TABLE II.

Limnodrilus.

	4th Day.	5th Day.	6th Day.	7th Day.	8th Day.	9th Day.	10th Day.	11th Day.	12th Day.	15th Day.	18th Day.
R	.625	.92	2.15	3.06	5.26	6.94	7.09	8.38	9.29	14.29	16.43
1*RS*	.031	.068	.093	.126	.150	.184	.213	.234	.212	.258	.221
10*RS*	.015	.034	.070	.099	.118	.167	.168	.201	.192	.273	.246
20*RS*	.003	.018	.047	.073	.108	.141	.145	.165	.163	.247	.227
30*RS*	.002	.008	.022	.043	.101	.112	.122	.110	.142	.207	.197
40*RS*			.011	.020	.058	.083	.097	.102	.123	.179	.174
50*RS*			.008	.014	.037	.048	.068	.072	.092	.159	.164
60*RS*				.008	.017	.025	.021	.052	.076	.109	.145
70*RS*					.008	.011	.012	.021	.037	.102	.143
80*RS*						.007		.014	.015	.052	.115
90*RS*								.008	.012	.012	.074

A copy of the averages from which the growth rate for the first and tenth regenerated segments was calculated. A partial revision of the averages for the total regenerated part is also included here. Measurements are in millimeters. *R* = length of regenerated tissue. 1*RS*, 10*RS*, etc., first, tenth, etc., regenerated segments.

Explanation of Table II.—Table II. is a copy of the averages, in millimeters, from which the growth rate for the first and tenth regenerated segments was calculated. A partly revised list of the averages for the total regenerated part is also included here. A comparison of Tables I. and II., especially considering the measurements of old segments in Table I., will give some indication of one disturbing factor in the polygon, *i. e.*, varying sizes or extension of the worms at the time of killing. *R* = total regenerated tissue; 1 *RS*, 10 *RS*, etc., = first, tenth, etc., regenerated segments.

the smallness and activity of the worms which made it impossible to cut precisely across or at the same level in the segments. Thus some cuts fell on the inter-segmental line, others at various distances within the segment, and several were diagonal. The

TABLE III.

Limnodrilus.

Day.	Epidermis.	Intestinal Wall.	Septa.	Muscle.	Nerve Cord.	Peritoneum.	Blood Vessels.	Chlorogogue.	Setæ.	Nephridia.
1	Old cells cover wound.	Proctodeum well formed. Chaotic.								
2	Proliferating.	Proliferating.	Columns forming.	Forming.						
3	Proliferating.	Proliferating.	Columns formed.	Distinct.	Anlage distinct.	Forming.				
4	Proliferating.	Little proliferation.	Well formed.	Distinct.	Distinct.	Forming.	Ventral present.			
5	Distinct, thin cuticle.	Little proliferation.	Well formed.	Distinct.	Well formed.	Well developed.	Ventral present.		Embryonic seta sacs distinct.	
6	Some mature.	Almost normal.	Well formed.	Distinct.	Well formed.	Well developed.	Both developed.	Present. Layer thin.	Embryonic seta sacs distinct.	
7	Some mature.	Almost normal.	Mature.	Mature.	Almost mature.	Mature.	Both developed.	Present. Layer thin.	Setæ well developed.	Anlage present.
8	Some mature.	Almost normal.	Mature.	Mature.	Almost mature.	Mature.	Both developed.	Present. Layer thin.	Setæ well developed.	One loop developing.
9	Some mature.	Mature.	Mature.	Mature.	Mature.	Mature.	Mature.	Present. Layer thin.	Nearly mature.	Immature.
10	Some mature.	Mature.	Mature.	Mature.	Mature.	Mature.	Mature.	Present. Layer thin.	Mature.	Immature.

last point seems to be especially important as Müller (1908) and others have shown that diagonal cuts produce marked differences in the detail of regeneration. (4) The state of contraction at time of killing was also probably somewhat variable so that detailed comparisons would have to be made with caution. The effort was made to test this factor by measuring the regenerated parts with the old segment as a unit. This indicates greatest speed of regeneration on the tenth day instead of the ninth as indicated by the other method. (5) The small number of specimens available gives opportunity for undue prominence in the showing of individuals. Evident extremes were omitted from the averages. But, after all, the general effect of most of these factors would be to diffuse rather than to accentuate the polygon, and there seems to be ample warrant for saying that the greatest speed of regeneration is at the ninth or tenth day, which is near enough for present purposes. In close approximation to these results we have those from some unpublished work by Mr. Frank L. Pinckney, using *Limnodrilus* in this laboratory in the spring of 1910. As he used the method of successive measurements of living animals, the similarity seems to be practically conclusive.

In examining the polygons two striking points appear which are rather difficult to explain. In the first place, there is an apparent slackening in growth almost to the zero point on the tenth or eleventh day, the most obvious explanation for which seems to be in an accidental assemblage of extreme characters. At any rate there was no change in temperature or other observable laboratory conditions at that time that could possibly account for it. In the second place there is a rise in the later portion of the polygon. This, however, in the total regeneration seems to be due to the fact that there are still some 50 or 60 very young segments, many of which are just at their maximum, thus serving to largely balance the decline of the older regenerated segments, which is not in itself, so very great by that time. As the study of tissue differentiation has been made principally on regenerated segment no. 10, the polygons for segments no. 1 and no. 10 are shown (Chart I.). The maximum for no. 10 appears here at the ninth day but is apparently only more or

less fortuitously coincident with the general maximum. Coincidence of this sort is hardly to be expected in all annelids. The abrupt rise for the tenth regenerated segment after the twelfth day is evidently due to accidental conditions in the groups.

For the differentiation study, longitudinal sections were used almost exclusively. All of these were sagittal or nearly so. While cross sections would certainly have given better histological detail they would have made it very difficult to interpret conditions in successive segments. The number of specimens favorable for sectioning was too small to allow the use of both kinds. The general features of the rate of differentiation in the regenerating part may be readily grasped by reference to Table III. The crookedness of some of the worms and the small size of all made interpretation of the sections rather difficult since so few sections in each specimen could be even approximately radial. There was the further difficulty, with many, of the fine sand in the digestive tract scratching and clouding the section even when it did not tear it. In spite of these disadvantages the characteristics of most tissues showed pretty well their relations to the embryonic and mature conditions. Ten tissues and organs are fairly easily distinguished in the mid body region of the normal worm. These will now be considered seriatim.

Mature epidermis is one layer in thickness with the form of cell varying from flat to cuboidal according to state of extension (Fig. 5). There is a well-developed cuticle. In regeneration there is first an extreme extension of epidermal cells in the neighborhood of the injury which in a few hours results in a covering of the cut surface. This extension is carried so far that the cells are quite thin and almost separated from each other. Then follows a period of very rapid proliferation in which the cells are three or four deep (Fig. 1). These cells are quite small, rounded, irregularly arranged and they have very little cytoplasm. This condition gradually changes until a portion four days old shows only a single layer. The cells even then are without well-marked walls, their nuclei are still prominent and they lack the definiteness so characteristic of the mature epidermal cell. A cuticle is easily distinguished by the fifth day and a sixth day epidermis is essentially mature though still staining a little differently and

lacking some indefinable quality of the old tissue (cf. Figs. 1–5).

Much the same things may be said about the intestinal epithelium. In this tissue cilia are well developed on the third day and it is evidently functional by that time but it retains some slight embryonic features to about the eighth or ninth day.

Septa are first distinguishable in the embryonic mass as radial columns of cells (Fig. 1) extending partly across the cœlomic space. They rapidly fuse into a continuous membrane reaching evident maturity by the fifth day (cf. Figs. 1–6).

The longitudinal muscle layer of the body is first noticeable as a very thin, somewhat scattering layer of long, spindle-shaped cells just inside the epidermis (Fig. 1). It does not change very definitely except for increase of thickness and compactness. Although otherwise well developed by the seventh day at latest, it is not of typical thickness until later (cf. Figs. 1–5).

The nerve cord is recognizable on the fourth day and almost mature on the fifth. It is apparently typical by the seventh day. Note in Fig. 5 the ventral localization of nerve cells in the cord so characteristic in this group of worms (cf. Figs. 3–5).

The embryonic peritoneum seems recognizable by location on the third day but is well developed by the fifth (Fig. 5), and in evident maturity by the seventh (Fig. 4).

The blood vessels were not easily followed. The ventral vessel is unquestionably well formed by the fourth day. Both were observed in some specimens of the sixth and both are certainly mature by the ninth (cf. Figs. 3–5).

The study of chlorogogue was very unsatisfactory. It would seem that it should show well soon after maturity of the intestinal wall at least and it is distinguishable on the sixth day and thereafter, but no specimen was found showing it in typical condition even at the tenth day. It is possible that the sand in the intestine affected its preservation more than that of the other tissues.

The setæ are easily followed even in toto mounts. The embryonic seta sacs are well formed on the fifth day, and the setæ with all their muscles seem to be functional by the seventh. There is no question of their maturity by the 10th day (Fig. 4).

Nephridia were rather difficult to identify. An irregular mass of embryonic nephridial cells was first located, mainly by position

on the seventh day. A single loop, probably functional, is plain on the eighth (Fig. 4), but the organ does not appear to be mature even on the tenth.

All organs and tissues except the nephridia appear to be functional on the eighth day. They have also reached apparent maturity by the tenth day, some before. The data are not as complete as could be wished but they certainly are very indicative of a correlation between the maximum rate of growth and the period of distinct differentiation. This is especially true when we consider the fact that the tenth regenerated segment is the one for which these data are recorded and that its maximum speed of growth falls on the ninth day.

Experiment II. An Immature Animal. *Amblystoma* Tadpole.

Material and Methods.

Eggs laid near Bloomington, Ind., probably in the night of January 29 were received in good condition at this laboratory on February 1. They were kept in small lots under similar conditions except variation in water, until it was found that they did very well in tap water, after which it was used regularly. Up to the time of hatching, the main efforts for control were in the line of change of water, keeping temperature below 20° C., and avoidance of direct sunlight.

On February 16, 100 tadpoles were selected from a quite uniform lot and placed singly in berry dishes with about 50 c.c. of tap water. On the average, these individuals were about two days from the egg, none more than four nor less than one. The dishes were partly covered to reduce evaporation. Water was changed and the dishes washed two or three times per week. Fresh water was frequently added between times if evaporation seemed excessive. The temperature record was taken twice daily. No efforts to feed were successful until February 20. Within a few days many were feeding readily on live *Limnodrilus* which was used continuously to the end of the experiment.

By March 11 several deaths had occurred but the larger number of tadpoles seemed to be well adjusted, seventy of the better specimens averaging about 20 mm. in length were retained,

TABLE IV.

Amblystoma Tadpole.

	Individual Number.	Mm. Removed.	Mm. Regenerated.
Immediate.....	70	5.75	
	69	6.	
	68	5.5	
	67	5.5	
1st hour.....	66	5.25	
	65	5.	
	64	6.5	
6th hour.....	63	7.	
	62	7.	
	61	7.	
1st day.....	15	6.	
	45	7.5	
	46	6.5	
2d day.....	16	6.	.152
	2	7.5	.152
	59	6.	.19
3d day.....	29	7.	.38
	14	5.5	.38
	32	6.75	.35
4th day.....	17	6.	.45
	47	7.	.76
	44	8.5	.91
5th day.....	28	6.5	1.14
	58	7.	died
	3	6.	1.03
6th day[1].....	33	7.	(.38)
	48	5.5	1.71[1]
	13	7.5	2.43[1]
7th day.....	43	7.25	1.90
	57	5.25	2.05
	18	6.5	1.37
8th day.....	27	6.5	2.28
	4	4.25	2.05
	19	6.	1.97
9th day.....	49	6.	2.28
	12	7.5	2.13
	26	7.	2.20
10th day.....	56	6.75	3.75
	5	6.	3.25
	35	6.	2.25
11th day.....	6	6.25	3.25
	41	7.	3.
	11	7.	3.75

TABLE IV.—*Continued.*

Amblystoma Tadpole.

	Individual Number.	Mm. Removed.	Mm. Regenerated.
12th day.....	55	7.5	3.5
	51	6.75	4.
	36	7.	4.
13th day.....	21	7.5	4.
	25	9.	4.
	24	7.	4.
14th day.....	54	7.5	4.5
	7	6.25	4.75
	37	7.	4.25
15th day.....	22	7.	5.
	39	6.25	5.
	38	6.	5.
16th day.....	8	7.	4.25
	23	4.5	3.5
	53	6.	4.5

[1] Killed five hours late. Deduct 20 per cent.

the others discarded. Operation was performed on March 16. Ten specimens were kept for check unoperated. Sixty had approximately one-half the tail removed. The anal opening and the tip of tail were taken as guide marks and the effort was made to cut one-half way between on estimate. It was also intended that the cut should be a right angles to the notochord. Of course, there were errors in both ways, some of the first being partly shown by the measurements in Table IV. The cut was made with a botanical razor against a block of paraffine. In order to steady the razor it was held at each end, the arms being rested on a table and the fingers on the paraffine block. Some of the error in judgment was due to some tails being more attenuate at tip than others.

Out of the 60 operated upon, four were killed immediately, three in one hour and three in six hours. The others were killed in groups of 3 on successive days from first to sixteenth inclusive. One of the three was taken from one end of the table the others from the other in order to avoid any possible influence of position. Gilson's mixture (Lee 1900) was used for killing followed by 70 per cent. alcohol, iodised 85 per cent. and the usual after procedure. Delafield's hæmatoxylin followed by picric acid gave

excellent results in staining, the muscles showing especially well.

Two adverse conditions must be kept in mind throughout the consideration of this experiment. (1) Lack of ability to control temperature evidently affected the rate of growth seriously as indicated by Chart II. (2) The old tissue is itself so embryonic in character that it is extremely difficult to make an accurate measurement of the amount regenerated or an adequate description of its changing features. Some of the vagaries of the polygon must be attributed to both these causes. General conditions were good as is shown by the fact of only one death in the regular series. Drawings are omitted because the requisite series is too complicated for a paper of this character.

Data.

These descriptions are somewhat indefinite because the facts themselves with few exceptions lack clear definition.

By the end of the first hour the epidermis had stretched over and very nearly covered the cut surface. In six hours it showed rapid proliferation, with cells closely and irregularly massed and three or four deep. This condition remained much the same in general appearance except for outward extension of the entire mass at the end of the first day. In two days the older regenerated epidermis was indistinguishable from the neighboring primary epidermis, though still proliferating at the tip. This general condition remains unchanged to the tenth day which is as far as the study of sections could be carried with any pretence of general accuracy.

The parenchyma showed some sign of reorganization in one day and there was a considerable apparent difference on the second day, but thereafter there was no appreciable difference from the ordinary conditions.

Bloodvessels were not in evidence in the regenerated tissue until the second day when some were seen which were evidently functional almost to the distal extremity of the new tissue. After the second day a fairly large vessel is uniformly found just beyond the distal end of the nerve cord, and some capillaries were identified quite near the tip in almost all specimens. Many of these were easily observable in the live specimens.

The first sign of extension of the nerve cord consists in a comparatively loose mass of nerve cells or nuclei at the extreme end on the first day. These have some appearance of migration. Little difference appears on the second day although a distinct lengthening of the cord has taken place. The third day shows nearly a half millimeter of regenerated nerve cord but the end shows no very definite sign of rapid growth. After that the condition cannot be distinguished from the ordinary.

The notochord shows some renewal in one day and a slight proliferation on the second after which it appears normal except for a very small area at the end which retains the proliferating character. This is true till about the fifth day when the distal one half to three-fourths of the notochord is markedly different in appearance from the rest. The cells are smaller and more strongly stained, even the cytoplasm taking the hæmatoxylin stain slightly. Careful study of the slide indicates that this distal part of the notochord is in a transitional stage of differentiation and that it has appeared by a simultaneous change in the new cells destined to form the distal notochord. It is interesting to note that whereas on earlier days the notochord had lagged behind the nerve cord it quickly became coterminal with and even extended slightly beyond the nerve cord by the eighth or ninth day. This peculiarity of notochord development is very strongly suggestive as it is coincident with the period of greatest speed in regeneration.

The first sign of muscle development is found on the sixth day when certain cell masses are recognizable as anlagen of the new muscle tissue. These are distinguishable through about one-half the extent of new tissue. On the seventh day a few proximal strands of muscle show fair development. And on the eighth day some are found throughout the proximal third. In nine days a few well developed fibers are distinguishable half way out in the new tissue and on the tenth day it is extremely difficult to identify the border line between the proximal regenerated muscle and the old tissue.

While most of the tissues studied reach the original state of development too soon to show any connection with or influence upon the highest speed of regeneration (Chart II.), it should be

remembered that all except the muscle are so generalized in their original condition that they require little change before reaching the original limit of differentiation. On the other hand, the muscle is rather highly specialized and if it be taken as a standard a rather marked correlation is noted. It is just becoming distinguishable at the sixth day when growth has its greatest speed according to this polygon, and it gradually takes on maturity until it is well developed by the ninth day which is evidently

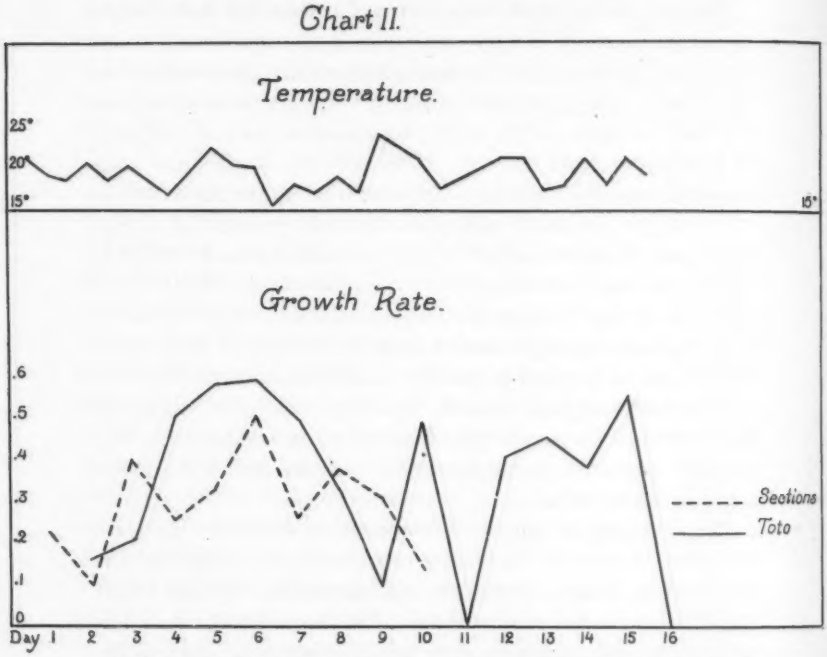


Chart II.—Temperature—1° C. to 1/20 inch rise. Growth rate—0.1 mm. to 5/20 inch rise.

somewhat past the time of most rapid extension. A glance at the chart indicates the probability of greatest speed having been shown at about the seventh or eighth day if the temperature had been under control.

Chart II. is a polygon constructed from micrometer measurements under the compound microscope, of the tissues in toto

TABLE V.

Amblystoma Tadpole.

Day.	Integument.	Parenchyma.	Blood Vessels.	Nerve Cord.	Notochord.	Muscle.
1	Proliferating.	Slight extension.		Some cell migration.	Slight renewal.	
2	Normal except. distad.	Some proliferation.	Functional almost to tip.	Slight extension.	Some proliferation.	
3	Normal except distad.	Slightly crowded.	Normal beyond nerve cord.	Normal, well extended.	Normal except at end.	
4	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	Normal except at end.	
5	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	Normal except at end.	
6	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	Normal except at end.	Traces through one-half regenerated part.
7	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	Normal except at end.	Some proximal fibers well developed.
8	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	One-fourth normal. Rest compact, heavy staining.	Some well formed cells, one-third distad.
9	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	One-fourth normal. Rest compact, heavy staining.	Some fibers one-half distad.
10	Normal except distad.	Normal.	Normal beyond nerve cord.	Normal.	One-fourth normal. Rest compact, heavy staining.	Some fibers one-half distad.

and after sectioning. The solid line indicates the former and the broken line the latter. Temperature fluctuations are recorded in the upper part. It was very difficult both with the toto examination and the section examination to distinguish the exact plane of cut, and of course, a very slight error in identification of this level would make a marked difference in the polygon. This difficulty rapidly became worse as the regenerated tissue grew older and measurements taken by any method after the tenth day have a purely suggestive value since they have no basis in strict accuracy. A set of measurements was taken with dividers, using a binocular dissecting microscope, but as their results are not essentially different they are not plotted on the chart. Table V. is self explanatory.

SUMMARY.

It seems that the results of these two studies are sufficiently clear to warrant assumptions as follows though further data are very desirable.

1. When adult tissue is removed the resultant regenerative growth reaches its greatest speed just preceding, or at least not later than, the time when the major somatic tissues become typically differentiated.

2. When immature tissue is removed the resultant regenerative growth rate shows no relation of any sort to the differentiation of the more generalized somatic tissues. There is, however, a marked correlation with such a highly specialized tissue as muscle, the differentiation of which is coincident with the period of maximum growth rate. And the peculiar development of the notochord also strongly supports this view.

Of the literature available, only that which was found distinctly useful is listed here. Krecker's list is good and a more general bibliography, especially concerning annelids, can be obtained from his paper.

PAPERS CITED.

Durbin, M. L.

'09 An Analysis of the Rate of Regeneration throughout the Regenerative Process. Journ. Exp. Zoöl., 3, 397–420.

Krecker, F. H.

'10 Some Phenomena of Regeneration in Limnodrilus and Related Forms. Zeitschr. wiss. Zool., 95, 383–450.

Lee, A. B.
'00 The Microtomist's Vade Mecum. London.
Minot, C. S.
'08 Age, Growth and Death. New York.
Morgan, T. H.
'01 Regeneration. New York.
Morgulis, S.
'07 Observations and Experiments in Lumbriculus. Journ. Exp. Zoöl., 4, 549–574.
'11 Regulation of the Water Content in Regeneration. Journ. Exp. Zoöl., 10, 321–348.
Müller, C.
'08 Regenerationsversuche an Lumbriculus variegatus und Tubifex rivulorum. Arch. f. Entwicklungsmech. d. Organismen, 26, 209–277.

ABBREVIATIONS.

R = regenerated tissue.
S = mature segment in old tissue.
RS = newly regenerated segment.
b = bloodvessel.
c = chlorogogue.
e = epidermis.
m = muscle.
n = nerve cord.
nr = nephridium.
pe = peritoneum.
pr = proliferating mass.
s = septum or septal anlage.
ss = seta sac.

Explanation of Plate I.

All figures are from camera drawings and partly diagrammatic. All have the same magnification (375 diam.). None are intended to represent cell detail. All show only most conspicuous features of the ventral side in or near the tenth regenerated segment.

Fig. 1. Third day. Septa almost formed. Muscles just distinguishable. Proliferation of epidermis and embryonic cells just inside muscle layer.

Fig. 2. Fourth day. Septa formed. Epidermal proliferation less. Cells massing to form nerve cord inside the muscle layer.

Fig. 3. Fifth day. Nerve cord, bloodvessel and peritoneum formed. No proliferation.

Fig. 4. Ninth Day. Typical ventral localization of cells in nerve cord.

Fig. 5. Old, mature segment. Note larger size, the thin epidermis and thick muscle layer.

Fig. 6. Eighth day. Optical section. Immature nephridium. Mature seta sac and muscles.

Fig. 1

Fig. 2

Fig. 3

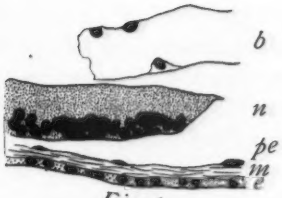


Fig. 4

Fig. 5

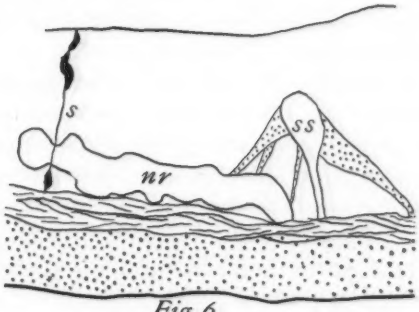


Fig. 6

W. E. ALLEN.

THE SMALLEST POLYODON.

THOMAS BARBOUR.

An article which appeared in a recent BIOLOGICAL BULLETIN by Danforth, entitled a "74 mm. Polyodon"[1] reminded me that some years ago I had seen some small specimens of Polyodon in the study collection of Fishes in the Museum of Comparative Zoölogy. A reëxamination of the material preserved there revealed three examples, the smallest hitherto recorded, as well as others which are among the smallest known. The three important examples are 35 mm., 37 mm. and 65 mm. long. The last individual was somewhat dried and was obviously similar in form and development to the specimen described and figured by Danforth. The smallest has been badly softened and swollen by immersion in weak alcohol. This individual was collected in Arkansas by Mr. G. Stolley and came to the museum before 1860. The other two small specimens as well as three more, which are 80 mm., 85 mm. and 93 mm., came from near St. Louis, Mo., and were collected in September, 1854, by a Dr. Eastman. They were in the private collection of Professor Louis Agassiz which was acquired by Harvard University as the foundation for the Museum of Comparative Zoölogy. These five examples have been kept in strong clear alcohol and are well preserved. The example of 37 mm. then is the important specimen in that it is the smallest known which is reasonably well preserved. Professor C. H. Eigenmann, who has seen this specimen, suggested that it was probably about three months old, in which case the egg laying would take place in July. If this were not really the case it is quite possible that Danforth's individual which was about 3 inches long was not the swift-grown young of the year but a specimen which had lived over one winter. Growth may be very slow or almost arrested during the cold months in the latitude of St. Louis. If, as is quite possible, the 37 mm. young was really much younger than three months, then this

[1] BIOL. BULL., XX., 4, 1911, pp. 201–204.

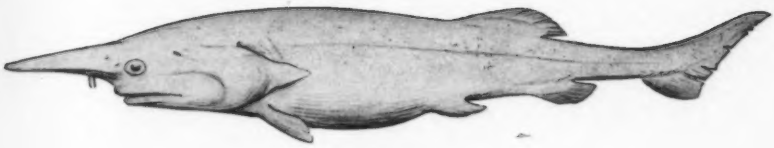
rate of growth would seem reasonable enough. Emphasis is laid on this point because great search has been made for the young during the months of spring and early summer. This search has been fruitless, while these inch-long fishes were got in September.

The drawings which have been made by Mr. E. N. Fischer with special regard to the proportions, show three aspects, dorsal, lateral, and ventral of the 37 mm. example, as well as of one 80 mm. long; two aspects of a fish 130 mm. long and a lateral view of a three-foot adult. These figures serve to show the remarkable features of the young as well as the changes in form which the species undergoes during growth, better than would a lengthy verbal description.

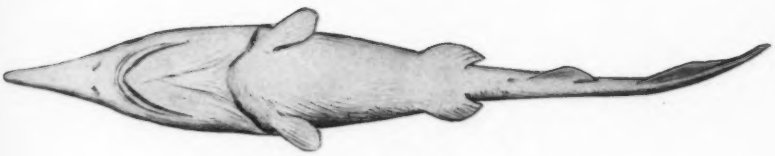
As may be readily seen from the drawings the most interesting characters to be observed in the young are the short sturgeon-like snout which has not yet become in the least spatulate; the extremely heterocercal form of the tail; the relatively long barbels and the less developed fins throughout. Unfortunately the notch near the outer extremity of the caudal fin is not shown n the very young because of the frayed margin of the tail fin. This character is marked in the slightly larger examples and, as is shown in the lateral view of the 80 mm. specimens, the notch marks the point where the cartilag nous fin rays cease to be developed. This character would seem to suggest that seen in the caudal fins of certain sharks where there is a similar notch. The whole general form of this, the smallest known selachostomous ganoid is certainly most strikingly shark-like as well as reminiscent of the adult forms of other ganoid genera.

EXPLANATION OF PLATE I.

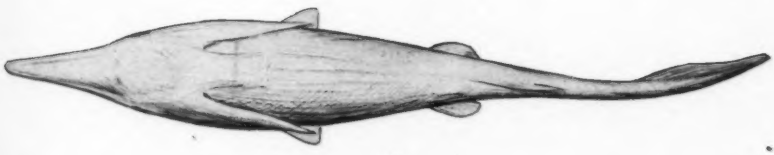
37 mm. Polyodon, × 3. Fig 1*A*, lateral view; Fig. 1*B*, ventral view; Fig 1*C*, dorsal view.



1A



1B



1C

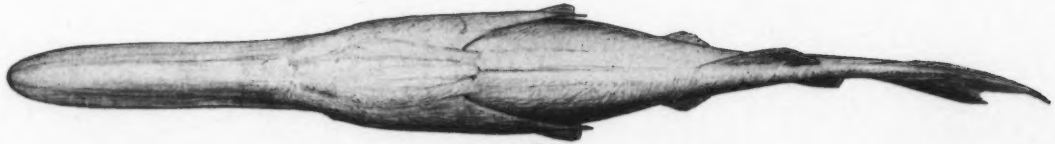
E. N. FISCHER DEL.

Explanation of Plate II.

80 mm. Polyodon. × 2. Fig. 2*A*, lateral view; Fig. 2*B*, ventral view; Fig. 2*C*, dorsal view.

2A

2B

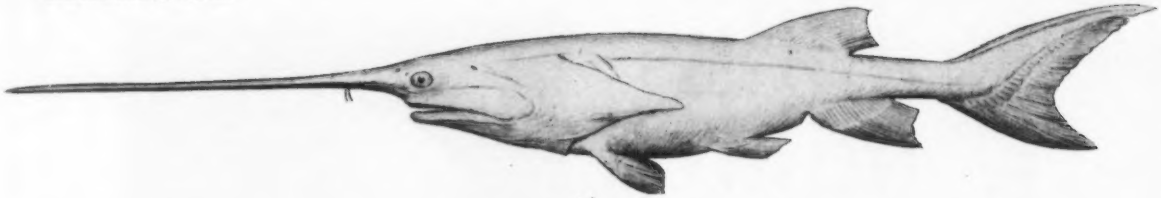


2C

E. N. FISCHER DEL.

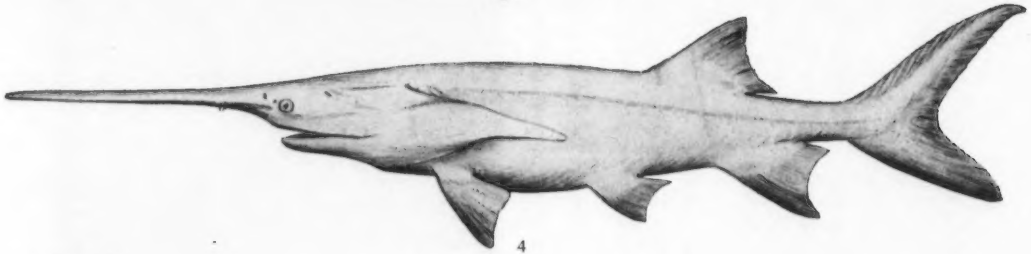
EXPLANATION OF PLATE III.

Figs. 3*A* and 3*B*, 130 mm. Polyodon, lateral and ventral views. ×1⅓. Fig. 4, 3 ft. individual from Boston market, 1/6 natural size.



3A

3B



4

E. N. FISCHER DEL.

NOTES ON TWO CROSSES BETWEEN DIFFERENT RACES OF PIGEONS.

T. H. MORGAN.

The present note is intended to record the results of a few crosses with pigeons. Through a series of accidents the experiment came to an untimely end. The breeding carried to the second generation has given too few individuals to warrant any generalizations, but enough to suggest the desirability of more extended work along the same line. As it will take two years to carry the crosses again to the second generation it seems worth while to record the facts so far obtained.

Cross Between a White Fantail and "Swallow."

A pure white female fantail (Fig. 1) was paired to a male

Fig. 1.

swallow (Fig. 2). One of the chief features of the breed of fantails is the large number of tail feathers; in the bird used here 32 feathers were present. I wished to find out how this

character would behave when brought into connection with the normal number (12) of tail feathers possessed by the "swallow."

FIG. 2.

The First Generation.—Seven young were reared from this cross. The tails of these hybrids had respectively 17, 12, 13, 15, 14, 13, 13 feathers. The result shows an intermediate condition be-

FIG. 3.

tween the parent types, with a distinct approach to the normal. The tail of the hybrid was not carried in the erect position as-

sumed by the fantail nor was it flat as in the other type, but formed a broad rather flat wedge like that of the fantail at rest, but not as high. It is shown in Fig. 3.

The F_2 Generation.—Only four birds were reared in this generation. Each had only twelve feathers in its tail. It may appear either that there is a further loss of tail feathers in this generation or that the normal number, twelve, represents the dominant type, and that the number of individuals is too small for the recessive type to reappear. Larger numbers must be obtained to settle this point, for, it seems not improbable that F_2 birds with more feathers than twelve are expected, even although the original number 32 may seldom or never reappear.

Other Characters.—It is not the object of this note to examine in detail the inheritance of other characters of these two breeds, but the following points may be mentioned. The fantail has a plain head (Fig. 1.), *i. e.*, no crest; the swallow has a crest. The F_1 birds all possess a crest. Some of the F_2 birds have a crest, others lack it. One bird that was killed is not recorded for crest.

The swallow has feathers on the tarsus and toes (Fig. 2), the fantail lacks these (Fig. 1). The F_1 birds have feathers on tarsus and toes, but less developed than are those of the father. Two of the F_2 birds lack feathers on the tarsus and toes, one bi d resembles the F_1 hybrid, and one has well feathered tarsus and toes. In the last case the feathers are as long, and as well developed, as in the swallow.

The fantail was pure white, the swallow has a dark top to the head, dark wings, and the feathers of the feet are light bluish. The wings are grayish blue and brown with two brown bars (Fig. 2). The tail, back, neck and ventral surface are white. The F_1 birds are pure white. Two of these birds showed two or three slate-colored feathers near the outer edge of the wings, generally to be seen only by brushing back the white feathers or when the wing is extended. Two of the F_2 birds are pure white, two were splotched, one with red, and one with brown. Only a small number of these colored feathers were present, but they were scattered unequally over the body and showed no tendency to be grouped on the wings and head alone.

[1] Owing to injury by rats the head was too much eaten to give certain records for the crest.

Summary.—The F_2 generation was inte mediate n number of tail feathers, feathers on legs, and color, although in regard to the last point the bi ds were practically l ke the white parent, a few feathers alone placing hem in the intermediate class. The crest was dominant. The F_2 generation showed segregation practically complete for the feathers on the legs, it showed segregation of white color to some extent, the recessive type appearing only to a very limited degree. The number of tail feathers showed a return to the normal, but the number of individuals is too small to lay much emphasis on the point. Yet it is significant that in all other directions the F_2 birds sufficed to show the influence of both parents.

Cross Between a Turbit and a Starling.

The F_1 hybrids of those birds were given to me by Mr. E. B. Southwick, who kept both parent stocks. The turbits (Fig. 4) were not very high-bred strains, but showed the main characteristics of their class. Their color is red or blue. The head is uniformly colored and has a cres : the wing bars are dark; along the middle line of the breast there is a series of reversed feathers (Fig. 4). The starling (Fig. 5) was a black bird with a white top to the head and white wing bars. On the breast there is a large light crescent with a metallic tinge. There are no reversed feathers on the breast.

The two hybrids (F_1) were red birds (Fig. 6). The crest was well developed. The top of the head was white (like that of the starling), but in one case not so sharply delimited. The wing bars were dark.

From these two hybrids eight, F_2 birds were reared. Five have crest and two lack the crest, five are red and three blue, all but one have darker bars on wing. T is one has a dark bar on most of the feathers of the wings but a few feathers have white bars.

Summary.—The most interesting result of this combination is the failure of the reversed feathers on the breast to reappear in the second generation. The result is like that of the number of tail feathers in the fantail-swallow cross, but here also the numbers are too small to make any conclusions possible, however

significant the absence of reversed feathers may appear. The *white* top of the head and the creast appear to be dominant; while the *white* wing bars are recessive.

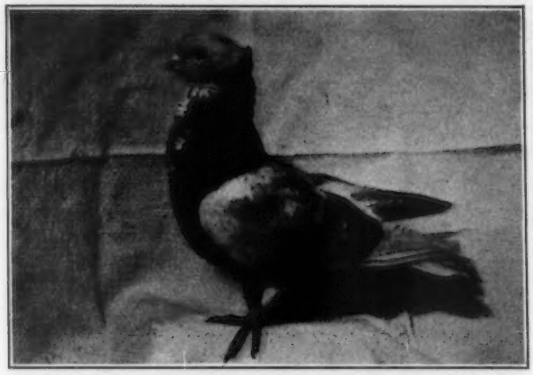


FIG. 4.

CONCLUSIONS.

As stated above the number of individuals reared is too small to warrant any extensive generalizations, but it is apparent that while certain characters show evidence of mono-hybrid inheritance, three characters do not furnish as good evidence. These three characters are the number of tail feathers in the fantail, the color pattern of the swallow, and the reversed breast-feathers of the turbit. It is probable that each of these characters has not been formed by a single step, but by a series of steps.[1] If this be granted it follows that the conditions present in the two races respectively can not be represented by a pair of Mendelian characters. There are then two interpretations; first, if the characters Mendelize there must be more than two factors involved; second the characters in question may come under some other kind of inheritance in which simple Mendelian segregation does not occur, or possibly the results may be partly Mendelian, partly not. A large number of F_2 offspring will be necessary before these points can be settled, but enough has been found, I think, to show at least in cases like these when a type

[1] See Morgan, T. H., "Experimental Zoölogy," page 171, New York, 1907.

has been formed either under domestication, or in Nature, not by one mutational step alone, but by a series of successive steps

FIG. 5.

along the same line, that the crossing with the original type or with a related type does not give Mendelian inheritance for one

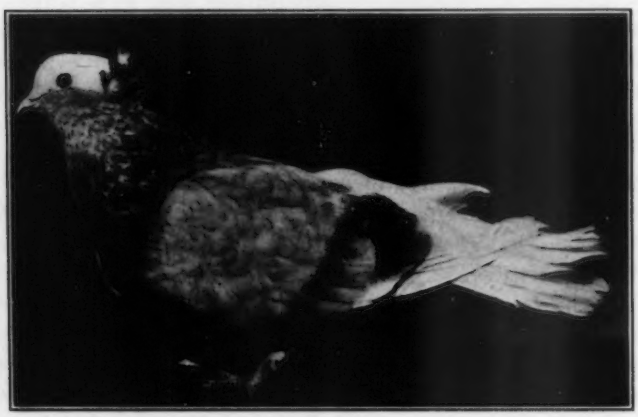


FIG. 6.

pair of characters. What we should expect under such circumstances we do not know as yet for we are now dealing with a

series of progressive or orthogenetic variations and not with a retrogressive mutation.

There is another result that seems to me to be especially significant. The white on the top of the head of the starling is dominant to the plain (colored) head of the turbit, but the white wing bars of the starling are recessive to the dark wing bars of the turbit. Unless these are two different kinds of white, one dominant (inhibitor of color) and the other recessive (absence of color) the result is difficult to explain, unless as I have suggested [1] the segregation in mosaic types is not germinal but ontogenetic.

[1] "The Influence of Heredity and of Environment in Determining the Coat Colors in Mice," *Annals New York Acad. Science*, XXI., July, 1911.

THE ŒNOCYTES OF PLATYPHYLAX DESIGNATUS WALKER.

WILSON P. GEE.

INTRODUCTION.

The œnocytes as unique cells of the body of all of the orders of insects which have been investigated, except the Aptera, have received much attention within the last couple of decades, but with relatively little more than mere conjecture as to their definite function. With the idea of throwing added light on the nature of the activities of these cells, the investigations here discussed were made on the larvæ, pupæ, and adults of *Platyphylax designatus*, a caddis-fly, which occurs quite abundantly in fresh-water springs in the vicinity of Madison, Wis.

The results here presented were derived from a study of the structure of the œnocytes in several periods of the life-history of this insect, and by injection experiments. Several stains were tried, Heidenhain's iron-hæmatoxylin proving the best for detailed work. Delafield's hæmatoxylin with orange G or eosin were the stains used in determining the location and number of the œnocytes. The cytoplasm of the œnocytes takes up eosin very readily, as it does also orange G. The substances used in injections were methylene blue as an intra-vitam stain and sulphindigotate of sodium to test the excretory nature of the cells.

The papers of Perez (1903), Rössig (1904), and Weissenberg (1907), presenting a rather complete review of the work done on œnocytes, it will here be necessary only to briefly mention some of the chief contributors to the subject.

According to Rössig (1904), the first to observe the œnocytes as special organs was M. Fabre (1856).

Landois (1865) distinguished them from the fat-body, and gave to them the name "Respirationszellen" because of the relation he observed them to bear to the tracheæ.

Haberlandt (1872) observed them in the silk-worm, where they were described by him as an organ of unknown function.

Graber (1873) separated the œnocytes sharply from the pericardial cells, and called them "eingesprengte Zellen." He said regarding their function: "Ohne Zweifel haben wir es hier mit eigenartigen einzelligen Drüsen zu thun, über deren secret allerdings nicht das Geringste bekannt ist."

The name "Oenocythen" was given to these cells by Wielowiejski (1886) on account of their resemblance in color, in the larva of *Chironomus*, to white wine. They were observed by him to be metamerically repeated under the hypodermis of the abdominal segments and arranged in small groups surrounding or near to the stigmata. This was the most extensive work on the œnocytes up to this time; for Wielowiejski was successful in identifying them in the Diptera, Coleoptera, Hymenoptera and Lepidoptera. He includes the œnocytes among the elements of the blood tissue and says regarding these: ". . . dass sie alle von dem sie umgebenden Medium gewisse Stoffe aufnehmen, zeitweise aufspeichern resp. verarbeiten und irgend welche Umsatzprodukte an dasselbe zurückgeben und dadurch auf die in den Hauptgeweben des Organismus vor sich gehenden Assimilations- und Desassimilationsprocesse einen Einfluss ausüben."

Kowalevsky (1887) attributed to the œnocytes a glandular function.

Verson (1891–2) in his work on the silk-worm gives them the name "cellule glandulari hypostigmatische," and finds their location exclusively under and behind the stigmata of the abdominal segments. As evidenced by the number of vacuoles in their cytoplasm during the larval stages, he attributes to them a glandular function. At the time of the spinning of the cocoon, two or three days before pupation, a second œnocyte generation appears in broad layers on the ventral part of the third, fourth, and the fifth abdominal segments. These attain a size of 60μ in the six-day pupa, and begin at this time to divide by amitosis. The larval œnocytes persist to the end of the life of the individual, reaching as much as 136μ in size.

Graber (1891) places the œnocytes with the blood tissue and gives their origin as ectodermal. He, however, considers them

as being metamorphosed into the fat-body, and also as giving rise to the blood corpuscles.

Wheeler (1892) in an extensive paper on the blood tissue of insects deals with the œnocytes at considerable length. He arrives at the following conclusions:

1. "The œnocytes are derived by delamination from the ectoderm just caudad to the tracheal involutions. They are also metameric organs.

2. "They are limited to the eight trachigerous abdominal segments.

3. "They appear to be restricted to the Pterygota, in all the members of which group they probably occur.

4. "They give rise neither to the fat-body nor to the blood but represent organs *sui generis*.

5. "After their differentiation from the primitive ectoderm, they never divide, but gradually increase in size."

With regard to their function, the only suggestion that Wheeler makes is in his discussion of their vacuolate structure in certain of the larvæ of the Trichoptera. Here he says: "One is reminded of certain gland cells which store up vacuoles of a specific substance in their cytoplasm, preparatory to secretion."

Heymons (1895) in the embryogenesis of *Forficula*, points out that the œnocytes are found in the eleventh abdominal segment as well as in the others. The eleventh segment bears no stigma, and thus they cannot bear any relation to the stigma as such.

Pantel (1898) classes the œnocytes as an organ because of their metameric repetition, and treats to a slight extent of their cytological changes in different periods of the insect's life. He hesitates, however, to commit himself as to their function, but rather inclines toward the excretory phases of their probable activities.

Berlese (1899), in his work on the ants, *Tapinoma erraticum* and *Pheidole pallidula*, finds that, in the beginning of metamorphosis, the larval œnocytes are multiplied, give up their fixed position, and, becoming amœboid, are scattered about among the fat-bodies. In his work on *Polistes gallica* and on the honey-bee, he finds a persistence of the œnocytes, with no special second generation. Their function he considers as excretory,

serving during the periods of moulting and pupation, when the Malpighian tubes are functionless.

Anglas (1900) describes the œnocytes as more or less amœboid, and with no special distribution in the Hymenoptera, except their existence in the abdomen alone. Except for a relative increase in volume, they remain throughout the life-cycle, in both distribution and structure, very similar to what they are in the early larval stages. He suggests that they perhaps secrete about themselves ferments, and considers them as glandular cells originating from the hypodermis and functioning as organs of internal secretion perhaps for dissociative purposes. The products of their secretion may serve for the general nutrition of the tissues of the body or even for the cytolysis of those larval cells destined to disappear.

Koschevnikov (1900), in his work on the fat-body and œnocytes of the honey-bee, finds that most of the large œnocytes of the larva disintegrate in the pupal stage; some of them continuing, however, into the beginning of the imaginal stage. In a young pupa, a second œnocyte generation, to which he gives the name "Imaginalœnocyten," arises from the hypodermis, and these œnocytes are later distributed among the fat-bodies. These are only one fifth as large as the larval œnocytes and are formed only in the pupal stage. He observed inclusions in some of the cells, and on this basis came to the conclusion that they are to be considered as excretory organs, without "Ausführungsgange," which serve as storage places for excess products of excretion.

Vaney (1902) says: "Chez la *Simulia*, le *Culex*, le *Chironomus*, durant la période nymphale je ne vois aucun changement dans ces cellules." However, he observes in *Simulium* that the number of œnocytes appears to have increased at the end of pupation. He concludes: "Chez les *Dipteres* les œnocytes ne subissent aucune histolyse durant la nymphose."

Perez (1903) states relative to the discovery of Koschevnikov, concerning the origin of a second œnocyte generation, that this opinion seems to be erroneous.

Rössig (1904) finds that, in the Cynipidæ, the œnocytes are limited to the abdomen. The larval œnocytes reach a length as great as 150μ and attain a proportionate length one fifth that

of the body. These larval œnocytes degenerate in the pupal stage. The imaginal œnocytes originate at about the same time through mitotic division of the hypodermal cells on the ventral side of the abdominal segments. They are described as very much smaller than the larval œnocytes, never equalling them in size.

Weissenberg (1907) finds in a well grown larva of *Torymus nigricornis*, over one hundred œnocytes, presenting no definite arrangement, and scattered about among the fat-bodies. They are in no way limited to the abdomen, nor do they necessarily have the definite tracheal attachments observed by Wielowiejski and others. The principal degeneration of the larval œnocytes occurs in the stage which he calls the "gelben Puppe." The larvæ shortly before pupation contain the imaginal œnocytes in the form of special œnocyte imaginal plates, which are laid in the niches of the dorsal hypodermis in the fifth to eleventh abdominal segments.

With regard to the function of these cells he says: "Schon oben habe i h mich gegen die Auffassung der Zelleinschlüsse als Exc ete ausgesprochen, möchte aber hier nicht das für Urate characterische Bild der braunen, radiär gestreiften, runden Krystallkörper darbieten, sondern an den frischen Zellen homogen und farblos erscheinen. '

Original Observations.

In the larvæ of *Platyphylax designatus*, the œnocytes are limited to the abdomen. A careful search through serial sections of the thorax of several individuals in different larval stages showed no traces of œnocytes in this portion of the body. They are in no way limited, however, to certain abdominal segments but occur in all, the larger number being present in segments two to seven. The definite tracheal attachments observed by many of the workers on this subject have not been seen in this insect. This observation agrees essentially with that of Weissenberg (1907) in *Torymus nigricornis:* "Von einer Befestigung an Tracheencapillaren, habe ich nichts bemerkt."

The œnocytes occur both dorsally and ventrally, by far the greater number occupying the latter position. The larger œno-

cytes, so conspicuous in the early larval stages, occur ventrally in pairs, one on each side of every abdominal segment. The smaller œnocytes of the earlier, as well as the later larval stages, are found principally in groups of from two to five, the larger groups appearing more ventrad. In several specimens they were observed near to or even grouped around a tracheal tube to which they, however had no connection. The location of all of the œnocytes is distinctly pleural.

The distribution of the œnocytes does not change essentially with the increase in size of the larvæ, but there is a less marked tendency for them to occur in groups. With the increase in size of the fat-body more of them become partially or completely surrounded by this structure. These changes are more marked in the late larval and in the pupal stages, but we frequently find in these periods, groups of as many as five œnocytes, and this tendency to grouping is observed even in the imaginal stage.

A careful count of the œnocytes of a larva eight millimeters in length totals about one hundred and twenty-five, these being approximately equally proportioned between the right and left sides of the body. From other estimates it is found that this number is about typical for *Platyphylax designatus*. There seems to be no wide fluctuation in numbers in different periods of the life-history, though the imaginal stages appear to show a decrease in number over the larval, due perhaps to disintegration. The conclusion that the number is constant for all the larvæ of the same stage, is, I believe, hardly justifiable, but there is no wide variation in this regard. In no case, have I seen evidences of division either by mitosis or amitosis in any of the stages studied, nor an increase in number in later larval, pupal or imaginal stages over that in the earlier larval periods.

While the œnocytes appear practically constant in number throughout the stages examined, there is to be noted a very marked increase in their size as the larva advances in growth. The following table represents the size of an average œnocyte derived from measurements of several of these cells in the stages indicated.

The largest size of the œnocytes is reached in the pupal stage; the greatest length of the nucleus proportionate to that of the

Length of Larva.	Length of Cell.	Length of Nucleus.
4.5 mm.	18.4μ	10.4μ
6 mm.	24 μ	12 μ
12 mm.	40 μ	20.6μ
22 mm.	77 μ	43.5μ
pupa	103.1μ	63.5μ
early imago	94.3μ	62.9μ

cell is found in the imaginal stage. There is to be noted, however, a decrease in the total length of the œnocytes of this latter stage, this being due, beyond doubt, to degeneration, manifest signs of which are present.

There are two sizes of œnocytes in the earlier larval stages. One of the larger type, from a larva six millimeters in length, is shown in the accompanying plate (Fig. 3). As already noted, these large œnocytes are present in each of the abdominal segments, ventral in position, one occurring on each side. These are many times larger than the œnocytes of the smaller type, and are pressed rather closely against the hypodermis. In the earlier stages of larval life, the greater comparative size makes these œnocytes very conspicuous, while in the older larva this is not so marked; in fact the smaller œnocytes in the oldest larvæ attain a size equal to or even greater than the formerly larger ones. Careful examination of serial sections of these larger œnocytes showed no traces of a duct leading from them.

The smaller œnocytes are more numerous than the larger. In the early larval stages, they are, as noted above, much smaller than the other type, attaining, in the old larvæ, an equal or greater size. Their distribution in the body is, however, very much more irregular.

In the earlier larval periods the œnocytes appear much more rounded in form than in the earlier stages. The nucleus shows the same tendency. The cytoplasm presents a finely granular, homogeneous appearance, and takes up rather evenly the orange G and eosin. In these stages, however, the cytoplasm appears rather more dense than in the succeeding periods.

Wheeler says with regard to the œnocytes of an unidentified phryganeid larva on which he worked that the nuclei contain no *nucleole*. In a 4.5 mm. stage of *Platyphylax designatus*, stained with safranin, a nucleole is seen to stand out very clearly (Fig. 1).

This apparently disappears in later stages. The bulk of the chromatin appears in small, rounded granules connected to form a skein. One easily observes several larger masses of chromatin standing out rather conspicuously in the nuclei of the œnocytes of all the larval stages. The nuclear membrane appears sharply defined in all of the stages except the imaginal.

Besides a relative increase in the size of the cell, there appears little change in the character of the œnocytes up to the last larval stage. At this period the nucleus becomes proportionately much larger and more irregular in outline. The cytoplasm shows, between the nuclear membrane and cell boundary, an inner and an outer zone or layer. The more central of these two layers shows the cytoplasm to contain more and larger vacuoles, and to have a denser appearance; the outer, smaller zone, on the contrary, contains fewer and smaller vacuoles. None of these vacuoles are clear, but one may distinguish in the larger ones, by careful focusing, many very small granules, which would seem to indicate the presence of more than a homogeneous content. These vacuoles have been observed by Verson (1891–2), Wheeler (1892), and others, all of whom interpret them as indicative of secretory activity. Wheeler (1892) says: "These vacuoles are but slightly refractive, and are not fat globules. This condition of the œnocytes was found in a number of larvæ, and I believe, represents a normal physiological state. One is reminded of certain gland cells which store up vacuoles of a specific substance in their cytoplasm, preparatory to secretion."

In the pupal stages, one finds that the nucleus has a more irregular outline, and that the cytoplasm contains comparatively few vacuoles. It is in this stage that the œnocytes attain their largest growth. This fact coupled with the appearances of activity in the late larval stages may perhaps be taken as indications of a greater secretory activity of these cells during these two periods.

In the imago, just after emergence, the œnocytes show marked signs of degeneration. The boundary of the cells has become indistinct and frayed, and the outer portion of the cytoplasm is stained rather deeply with the hæmatoxylin (Fig. 7). At this stage, the nuclear membrane is not distinguishable, and but few

vacuoles are to be noted in the cytoplasm. The chromatin has gathered into irregular masses along the sides of the adjacent cytoplasm (position of former nuclear membrane) and the linin appears in masses which have separated from the chromatin material. In an imago two or three days after emergence, but which had been kept enclosed in a small dish, the degeneration of the nucleus is seen to have proceeded further. The cell does not, however, present an essentially different appearance to that shown in the imago two or three hours after emergence. This third-day imago was the latest stage of which material was available. It would be interesting to determine from older imagoes whether the œnocytes entirely degenerate and are taken up for the purpose of general nutrition of the tissues, thus affording a reserve food supply in addition to their secretory function. This latter view would, however, be hard to understand considering the short life of these insects, and also the fact that egg-laying goes on shortly after emergence from the pupal case.

Various functions have been attributed to the œnocytes. Landois (1865) gave to them the name "Respirationszellen" and assigned to them a place in the respiratory system. Graber (1873), in his early work, speaks of them as unicellular glands, the secretion of which was of unknown function; later, however, he holds that they are metamorphosed into the fat-body, and give rise to blood corpuscles. Berlese (1899) considers the œnocytes as excretory in function, as does also Koschevnikov (1900).

Anglas (1900) says: "Ce fait permet d'affirmer qu'ils sécrètent autour d'eux des ferments. Aussi les considérons-nous comme des cellules glandularies (nées de l'hypoderme) et jouant le rôle de glandes à sécrètion interne, mais de glandes dissociées." Verson (1891–2) considers them as secretory in nature as do also Rössig (1904) and Weissenberg (1907).

Holmgren (1900) in *Apion flavipes*, experimenting by injecting small quantities of powdered methylene blue and alizarin-cyanin into the body cavity, came to the conclusion that they were excretory in function. He found that the œnocytes took up the color in much the same manner as the Malpighian tubes, the nucleus of the œnocyte showing signs of staining somewhat earlier than the nuclei of the latter organs. Three hours after injection,

he found that the methylene blue had passed into the lumen of the Malpighian tubes in sufficient quantities to color them; the œnocytes at the end of an hour and a half had lost their color.

Following out this line of investigation, a dozen or more specimens of *Platyphylax designatus* were injected with an aqueous solution of methylene blue and the parts dissected and removed to a glycerine mount. It was found that immediately after injection the œnocytes and the spinning-glands, so prominent in this form, had both taken up the stain—the œnocytes to a much less marked degree than the latter. In only one instance, was it found that the Malpighian tubes had taken up the methylene blue, and in this case the color was seen in but a single tube. The œnocytes took up the stain more in the nucleus than in the cytoplasm, the latter, however, being distinctly tinged. The larvæ killed a half hour after injection showed the œnocytes more deeply stained, but no coloration was observed in the Malpighian tubes. Larvæ killed one hour after injection showed that the Malpighian tubes had begun to take up the blue color, but that the œnocytes and spinning-glands at this time were becoming less intense in their coloration. These results would lead one to rather different conclusions than those reached by Holmgren. It would be inferred from the similarity of the reaction of the œnocytes and spinning-glands as detailed above, that the former are secretory though of a different nature and intensity to that of the spinning-glands.

This view is further strengthened by injection experiments with sulphindigotate of sodium. Fürth (1903) says: "Die exkretorische Bedeutung der Malpighi'schen Schläuche offenbart sich auch in unzweideutiger Weise durch ihr Vermögen die Ausscheidung von Farbstoffen zu bewerkstelligen." Schindler (1878) found by injection of a solution of sulphindigotate of sodium into the body cavity of *Gryllotalpa* that the Malpighian tubes excreted the substance within twenty-four hours.

An aqueous solution of sulphindigotate of sodium was injected into the abdomen of a dozen or more larvæ of *Platyphylax designatus* 15–20 mm. in length, the insertion of the hypodermic syringe being ventral and thoracic, but the solution extending thoroughly

into all the space between the hypodermis and the digestive tract. Specimens killed within an hour after injection and mounted as in the former experiment, showed a slight accumulation of the solution in the lumen of the Malpighian tubes. This was even more marked two and a half hours and four hours after injection, a diffuse blue appearance being then very apparent throughout the cells of most of the tubes. The œnocytes, however, showed no signs of coloration at any of the periods observed nor any evidences of excreting the substance injected.

Just what the function of these peculiar cells may be it is difficult to say. In no case have I observed in the cytoplasm of any of the œnocytes what could be interpreted as accumulations of solid products of excretion. Their behavior toward methylene blue, and sulphindigotate of sodium would seem to show that they are secretory rather than excretory in function. The nature of their secretion is difficult and practically impossible to determine. Measurements show their greatest size in *Platyphylax designatus* to be in the pupal stage, and evidences from a purely structural basis seem to point towards the greatest functional activity during the late larval and the pupal stages. Their intimate relation to the fat-bodies seems vastly more significant than the occasional grouping about a tracheal tube. Their relative increase in size, while of course proportionate to the increase in size of the body, seems to bear a direct relation to the size and development of the fat-bodies. It is also found that their identity is retained throughout the period of metamorphosis. Can it be that their function is the secretion of a substance or enzyme which is of aid to the fat-body in its constructive work or is it that their function is, as Anglas (1900) suggests, that of internal glands of a dissociative nature, their secretion serving to aid in the elaboration of products for general nutrition of the tissues or for the disintegration of larval cells destined to disappear? These are very pertinent questions, but ones to which a definite answer cannot as yet be given. It seems safe to conclude that they are internal, ductless, secretory glands, whose function may, in the light of recent progress in the field of internal secretion, be for a long time a matter of conjecture.

These investigations were taken up at the suggestion of Prof.

W. S. Marshall, to whom I am indebted for many suggestions in the direction of the work.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF WISCONSIN.

REFERENCES.

Anglas, J.
'00 Observations sur les métamorphoses internes de la Guèpe et de l'Abeille. Bull. Soc. France et Belgique, XXXIV.

Berlese, Antonio
'99 Osservazione su fenomeni che avvengono durante la ninfosi degli insetti metabolici. Riv. Patalogia vegetale, Anno 8.

Fabre, M.
'56 Étude sur l'instinct les métamorphoses des Sphégiens. Ann. Sc. nat. (3 ser.), Zool. VI.

von Fürth, Otto
'03 Vergleichende chemische Physiologie der niederen Tiere. Gustav Fischer, Jena.

Graber, V.
'73 Ueber den propulsatorischen Apparat der Insecten. Arch. mikros. Anat., IX.

Heymons, R.
'95 Die Embryonalentwicklung von Dermapteren und Orthopteren unter besonderer Berücksichtigung der Keimblätterbildung monographisch bearbeitet. Jena.

Holmgren, Nils
'02 Über die Excretionsorgane des Apion flavipes und Dacytes niger. Anat. Anz., XXIII.

Koschevnikov, G. A.
'00 Über den Fettkörper und die Oenocyten der Honigbiene (Apis mellifica). Zool. Anz., XXIII.

Kowalevsky, A.
'87 Beiträge zur Kenntniss der nachembryonalen Entwicklung der Musciden. Zeitschr. wiss. Zool., XLV.

Landois, L.
'65 Ueber die Function des Fettkörpers. Zeitschr. wiss. Zool., XV.

Pantel, J.
'98 Le Thrixion Halidayanum. La Cellule, XV.

Perez, Ch.
'03 Contributions à l'études des métamorphoses. Bull. Soc. France et Belgique, XXXIX.

Rossig, H.
'04 Von welchen Organen der Gallwespenlarven geht der Reiz zur Bildung der Pflanzengalle aus? Zool. Jahrb., XX.

Schindler, E.
'78 Beiträge zur Kenntniss der Malpighi'schen Gefässe der Insekten. Zeitschr. wiss. Zool., XXX.

Vaney, C.
'02 Contribution à l'étude des larves et des métamorphoses des Diptères. Ann. Univ. Lyon (nouv. ser.), Sc. Med., Fasc. IX.

Verson, E.

'91 Cellule glandulari ipostigmatische nel Bombyx mori. Boll. Soc. entomol. Ital., Anno XXIII.

'92 Altre cellule glandulari (epigastriche) di origine postlarvale. Richerche Anat. Staz. Bacol. Padova, VII.

Wheeler, W. M.

'92 Concerning the blood-tissue of insects. Psyche, VI.

Weissenberg, R.

'07 Önocyten von Torymus nigricornis Boh. Zool. Jahrb., XXIII. (Anat.).

Wielowiejski, H. R.

'86 Über das Blutgewebe der Insekten. Zeitschr. wiss. Zool., XLIII.

Explanation of Plate.

All figures drawn with a camera-lucida. × 630.

Fig. 1. Œnocyte from a 4.5 mm. larva.

Fig. 2. One of the smaller œnocytes from a 6 mm. larva.

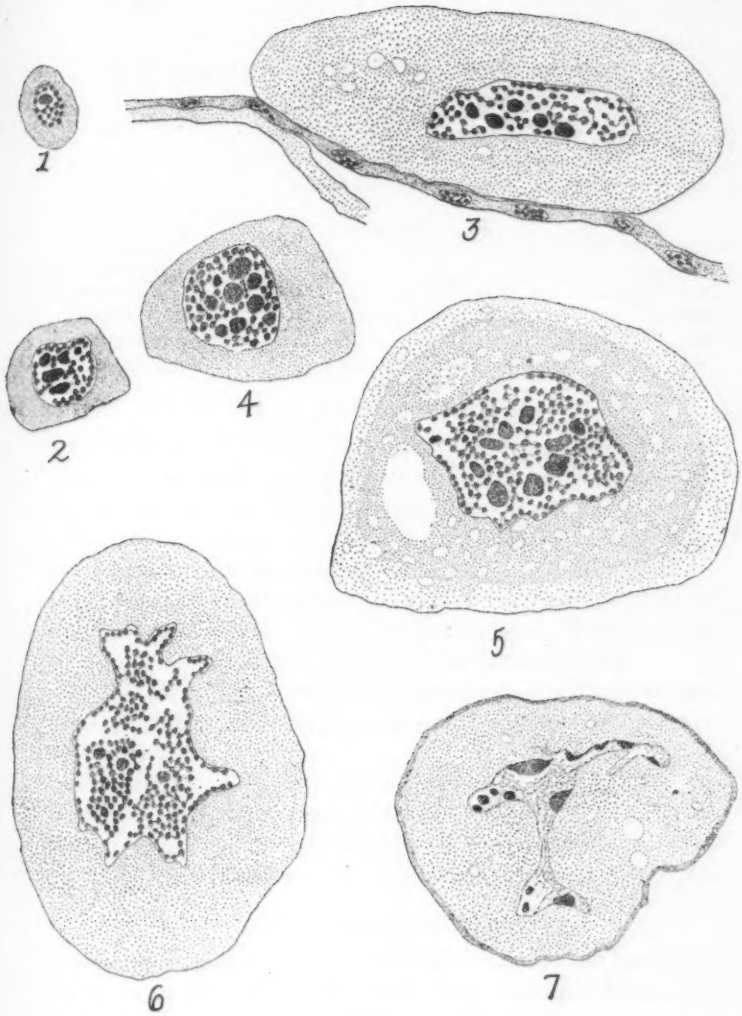
Fig. 3. One of the larger œnocytes from a 6 mm. larva.

Fig. 4. Œnocyte from a 12 mm. larva.

Fig. 5. Œnocyte of late larval period showing vacuoles in cytoplasm.

Fig. 6. Œnocyte of pupal stage.

Fig. 7. Œnocyte from newly emerged imago, showing signs of degeneration.



WILSON P. GEE.

THE MATING HABITS OF FOUR SPECIES OF THE BRACHYURA.

F. E. CHIDESTER,

CLARK UNIVERSITY.

CONTENTS.

		Page
1.	Introduction	235
2.	Observations and experiments	235
3.	Mating habits of other Arthropoda	240
4.	Conclusions	245
5.	Bibliography	246

1. INTRODUCTION.

In the course of a study of the general activity of the crayfish which has occupied my attention for the past three years, I became interested in the mating habits of Arthropoda.

During the summer of 1910, while acting as a temporary assistant in the Woods Hole laboratory of the Bureau of Fisheries, I had the opportunity to observe the mating habits of four species of marine crabs, and to experiment with them to determine if possible their manner of sex discrimination.

In this paper I shall briefly describe the results obtained and consider similar work done by various investigators on the other Arthropoda.

Acknowledgment is gratefully made to the Hon. Geo. M. Bowers, U. S. Commissioner of Fisheries, for the opportunity to work in the Woods Hole laboratory; and to Professor J. P. Porter of Clark University for reading and criticising this paper.

2. OBSERVATIONS AND EXPERIMENTS.

The species observed were: *Callinectes hastatus*, the blue crab; *Cancer irroratus*, the common rock crab; *Carcinus mœnas*, the green crab; *Platyonychus ocellatus*, the lady crab.

A description of the differences between the male and the female; the virgin female; the ovigerous female and the ovigerous female about to lay, will apply in general for all of the species.

In the male the abdomen is narrow and the intromittent organs or verges are the first pair of abdominal appendages. The paired penes are formed by the everted posterior parts of the efferent ducts. The penes transmit spermatophores down the grooves of the verges into the seminal receptacles of the female.

In the virgin female the abdomen is much like that of the male, except that the telson is narrower. Hay (15) discovered the presence of a pair of hooks which project from the body and fit into a pocket on either side of the abdomen, holding the abdomen tightly flexed against the ventral surface of the carapace. The swimmerets are very small n the virgin female. Crabs moult several times during the summer, according to Herrick (16).

At the moulting the broad abdomen of the ovigerous female is liberated from its narrow quarters in the old virginal shell, and the female is ready for impregnation. The abdomen of the ovigerous female is held flexed only by muscular contraction.

A few days after impregnation, the female may be seen to have two spermatophore plugs projecting slightly from the vaginæ and effectually closing the entrance. These plugs are probably secreted by the glands of the male. Andrews has found that the spermatophores of the crayfish are held in the annulus ventralis of the female by plugs secreted by the glands of the male (2). It is not known by me just when the plugs are forced out from the vaginæ of the female crab, but probably they are retained until just before the laying. This would be the case if the crabs are to be classed at all with the crayfish in which fertilization is external, and in which the annulus ventralis acts as a *temporary* sperm receptacle.

SPECIMENS STUDIED.

	Males.	Females. Virgin.	Females. Ovigerous.	Females. With Eggs.
Blue crabs	3	3		
Rock crabs	3	2	7	1
Green crabs	2	2	2	
Lady crabs	2	2	2	

In the above table the virgin females are those that were brought into the laboratory and moulted there.

Distinction between the ovigerous females showing spermatophore plugs and those not showing them will be made as the experiments are discussed.

The crabs were kept in a sea water aquarium 2 × 2½ × 4 feet. In the case of the blue crabs, observations and experiments were made only on those confined in this tank. The other species were smaller and individuals were from time to time removed to circular bell jars, 9 in. high and 9 in. in diameter, with about 6 in. of sea water in them.

Blue Crabs.—The female about to moult is seized by a male and carried about by him for a day or two until she is ready to shed her shell. During the pre-moulting period the female is in a normal upright position, being held by the second, third and fourth pair of ambulatory appendages of the male. It is stated by Hay (15) that the male appears able to distinguish the female which is about to moult. I was not able to test this in the blue crabs, but from the observations on the behavior of the rock crabs, green crabs and lady crabs, I am inclined to question the statement and to believe that the male is not able to distinguish the female about to moult except from the fact that she is less resistant. I have noted this sluggishness and apparent weakness in the crayfishes for several days before moulting (7).

When the female is in the process of moulting, she is left for a time by the male. Then he approaches her and turns her over so that her head is beneath his abdomen. I was unable to see the first approaches of the male to the soft-shelled *ovigerous* female, but separated two pairs many times and watched the readjustments.

When the female has been turned so that her ventral surface is apposed to that of the male, and her head is situated beneath his abdomen, both remain quiet for several minutes unless disturbed.

After a period of from thirty seconds to ten minutes, the male taps the female lightly with his first and second ambulatory appendages and turns her slowly around underneath him. The male is not, however, the only active party. No sooner has the female been aroused from her semi-hypnotic state by the movement and tactile stimuli of the male, then she begins to use her ambulatory appendages in turning herself. In order to determine if an external stimulus would cause an early adjustment, I tapped lightly on the edge of the thoracic region of the female, by means of the tip of a small seeker. This was tried on three

successive occasions and in each case the female began to move herself into the copulating position by walking around the legs of the male, placing her legs between and against his. In one of these cases, the female moved so rapidly that the male may have thought she was attempting to escape, for he beat upon her carapace in the attempt to quiet her.

It is extremely interesting to see such activity on the part of the female crab, when one has as a contrast the passivity of the female crayfish.

When the female crab has turned about two thirds of the way around, adjustment of the abdomens must be made. As above stated, the abdomen of the ovigerous female is not bound by hooks to the body and is held flexed by muscular contraction. During the preliminary maneuvers, the abdomen of the female is held slightly extended, and the male must push this partially extended obstacle out still farther and interpose his own telson between it and the body of the female. In the case of the blue crabs this is not difficult. The verges of the male are inserted in the seminal receptacles of the female, and copulation takes place. The male carries the female about, grasping her with the tips of his ambulatory appendages, and holding her for hours, sometimes days. While the act of copulation is going on, the female is exceedingly quiet. The male is very pugnacious. Bachelor males were placed in the vicinity of a mated pair of blue crabs and were beaten away by the vigorous defense of the male in copula.

The two male blue crabs under observation were tested as to their recognition of females, hard and soft, of the three other species. In no case did a male attempt to copulate with an alien female.

Rock Crabs, Green Crabs and Lady Crabs.—These species are all smaller than the blue crabs, and are not materially different in appearance from each other. In the case of the blue crabs, I did not succeed in getting a female with spermatophore plugs. In the case of the three species under present consideration, I did secure several ovigerous females that had been fertilized before they were brought into the laboratory, and also secured a rock crab with eggs.

The method of capture of the female by the male is the same in these crabs as it is in the blue crabs. The ability of the blue crabs to distinguish by sight, however, seems to be greater. In handling the members of the different species, one is struck immediately by the remarkable visual powers of the blue crab. He is alert to moving objects and knows when a body comes within striking distance.

Males of all three species were tested by removing the soft females and replacing them by hard males. They attempted in all cases to copulate with the hard males. Then in the case of the rock crab, two males were tested by placing with them, hard, ovigerous females, in which the spermatophore plugs were not protruding. In these cases, the males succeeded in inserting the verges and actual copulation took place.

In the case of the males with whom were placed other males, the approach of the recently copulating male to the stranger male was made in a similar manner to his approach to the lost partner. When the stranger resisted violently, the other male attempted to beat him into passivity just as he successfully did with the females, hard and soft. Just as I have previously held in the case of the crayfish, I now hold that the ability of the male crab to distinguish the sexes is really a matter of inability to hold and copulate with the male individual. It seems that discrimination is tactual and kinæsthetic.

Among the females captured for me were four ovigerous rock crabs, two ovigerous green crabs and two ovigerous lady crabs, all of which had evidently been fertilized some time before, for they were successfully protected from a second fertilization. For about 3 mm. around the apertures of the seminal receptacles, the shell was soft. In the openings of the vaginæ were seen two grayish vermicular plugs. These were pulled out, the operation evidently causing discomfort to the female. The plugs were curved in such a manner that they appeared to have been moulded in the vaginæ. At the internal ends were the spermatophores, emptied and flat. In order to determine if these spermatophore plugs were formed before they were visible from the outside, I dissected a female which had been killed two days after copulation, and found that the plugs were present, but that the hardened outer ends were not protruding.

Experiments were made with males of the three species under consideration by placing with them females of the same species known to be fertilized and to have protruding plugs. In no case was a male successful in his attempts to copulate. In the case of one rock crab, the female was beaten into submission and the male succeeded in touching the plugs. The discomfort caused such violent struggles on the part of the female that she was released.

The female rock crab with eggs was placed with a male of the same species, but his efforts to overcome her resistance were futile. In neither the fertilized and spermatophore plug-bearing female, or the egg-bearing female is a true passive state produced by the beating of the chelæ and ambulatory appendages of the male.

In the case of a large male and a small female, the adjustment of the abdomens is very difficult. In the case of one large male it was noted that the adjustment was not possible in the normal position, and that he turned upon his back so that the female floated above him and her abdomen was extended to a greater degree. Then he slowly drew her into position, and having inserted his verges, assumed the normal position above her. In all of the species studied, the female is not passive in the later stages of adjustment, but uses her ambulatory appendages to assist in the turning of her body. I should have liked to place a soft-shelled male with a male that had just been separated from a soft female, but was not able to do so.

3. Mating Habits of Other Arthropoda.

Crustacea.—Herrick did not study the mating habits of the lobster (16) but noted the fact that there were external seminal pouches in the female. He states that the copulation is independent of the moulting of the female. Herrick mentions the discovery by Milne-Edwards of a female *Cancer pagarus* which was fertilized and in which were what Milne-Edwards at first took to be the wands of the male, broken off after copulation as in the insects. Later, Milne-Edwards said in reference to this observation that it seemed probable that the stoppers found in the copulating pouches of the female were "of the nature of spermatophores rather than a fragment of the penis."

Holmes (17) found that in Amphipods the oviposition occurs after the moulting of the female. The males capture the females before the moult and leave them for a few hours, resuming possession of them after the moult. He found that the method of recognition is neither by sight nor smell. He removed antennæ and blackened the eyes, and still the males grasped the females. Holmes concludes that the male has the instinct to seize and carry another individual of the same species, while the female has the instinct to lie quiet when seized. He found that dead individuals were not carried and explained this by the lack of an occasional movement causing a struggle on the part of the male to retain his hold. Holmes has mentioned also the work of Leydig on *Artemia*, in which the latter found that the males of *Artemia* clasp the females by means of peculiarly shaped antennæ, and the two sexes swim about together for several days.

Dohrn (Holmes, 17) noted that the males of copepods while mating, swim about on the backs of the females much as in the Amphipoda.

Dearborn (9), Andrews (2, 3), Chidester (6, 7) and Pearse (26), working with crayfish, have shown that recognition is largely tactual. Andrews discovered the annulus ventralis in *Cambarus*, the American crayfish found east of the Rockies. This serves to retain the spermatophores until the female lays her eggs. In the crayfish, fertilization of the eggs is external. Chidester found (6) that males grasp other males and attempt to copulate with them. Pearse found that males attempt to copulate with dead females of the same and different species (26). Andrews holds that the male recognizes the female as a female because she does not resist, hence the dead or bound male is taken for a female. He says (3): "It is hus possible that from the crayfish standpoint the only difference between the sexes is a difference in behavior and not a difference in form, and moreover a d fference received by muscle and touch sense and not in effect upon any other sense organs."

It seems to the writer, that although in the crayfish and the lobster, he males do not capture the females and watch over them while they moult, the process is analogous, for the freshly moulted female is soon captured and fertilized. In the case of

the crayfish which have annuli, the subsequent copulations are futile on account of the presence of the hard vermicular plug deposited in the false pouches by the male. Again, in the lobster and in the European crayfish, *Astacus*, although there are no pouches, the spermatophores would naturally adhere to the freshly moulted surface better than to a surface covered with accretions.

The term "recognition" has been used by Pearse and others without a proper appreciation of the fact that we have absolutely no right to assume a true r cognition in the Arthropoda. Andrews has brought out this fact in his statement regarding the crayfish, *Cambarus affinis*. He says (3): "Sex 'recognition' exists apparently, only in the sense that the male may carry out all the stages of conjugation if a female happens to be seized, but not if a male is seized."

"Recognition," it seems to the writer, should be relegated to disuse in speaking of the lower animals. The term "discrimination" may be safely used. Arthropoda may use vision, "contact-odor," kinæsthetic or other senses in *discrimination* without really "recognizing" sex.

Insecta.—Feré, in a study of cockchafers (11) found that sexual coupling failed to take place when the antennæ were removed. He also found that males which had just coupled with females proved sexually attractive to other males. It is not just certain that Feré was correct in supposing that the males were attractive to other males, because the former retained the odor femina. He found similarly in *Bombyx* that males sometimes proved attractive to other males (12).

Mayer, working with moths (21), found that the male moths find the females by smell (Forel's contact odor sense?) rather than by sight. Males flew to the abdomens of females which were placed near the detached winged bodies. Males would pair with a female minus wings, but would not approach a male with female wings. In the moths, there would seem to be a distance-odor perception.

Berlese observed that in flies, there was only a slight activity of the external organs of the male, and a great activity of the female. The male mounted the female, but the female was then

the active party, introducing her ovipositor into the genital atrium of the male (5).

Maeterlinck's popular description of the life of the bee, states that the male honey bee captures the female in her nuptial flight and leaves part of his copulatory appendages in her seminal receptacles (20). The factor of natural selection is active in the mating of the bee, for the strong males are the ones to capture the females.

Fielde (13), Wheeler (34) and others mention the mating of ants. Sex-discrimination is by smell. Forel calls it "contact-odor."

Mitchell (22), worked with mosquitoes, finding that the sexes discriminate each other by the song. The female flies into a cloud of dancing males. It is possible that discrimination is *visual* at first, rather than auditory as Miss Mitchell believes.

In confinement, the female stands quietly on the floor of her cage with the male beneath and clinging with his body parallel to hers. Any disturbance which causes the female to move, when the pair are not copulating, causes the male to seize her again. The mating is repeated every hour or oftener, when the male is excited by a movement of the female. This may be brought about by a tap on the cage. When unconfined, one male unites with several females. A single fertilization lasts for five layings. Miss Mitchell concludes that the male continues to approach the female by means of the olfactory stimuli through the sensitive hairs on the antennæ. Distant localization is according to her idea, by sound; discrimination of the quiescent female near at hand is by smell. Moulting as occurring before fertilization is not mentioned.

Stockard, working with the walking-stick, *Aplopus Mayeri* (32), found that males mated with artificial females made by fastening the abdomens of females to sticks and supporting the sticks on wire legs.

Arachnida.—Montgomery, 1903 (23), and Porter, 1906 (30), have summarized pretty fully the investigation made on the mating habits of spiders. Both these writers have contributed much original investigation. I shall not attempt to give all of the literature, relative to the mating habits of spiders, but content myself with a brief summary of the more important work.

The Peckhams have taken up in detail the courtship of spiders, and hold that (27) in the spiders, Darwin's theory of sexual selection is upheld.

Emerton (10) finds some variation in the ferocity of th. female spiders. He states that in *Linyphia* and *Theridion*, the male and female live peaceably together in the same web and the male is not attacked by the female during his approaches.

In *Agalena* the male s the stronger of the two. He takes the female in his mandibles and lays her on one side and inserts one of the palpi; then he turns her so that she lies on the other side with the head in the opposite direction and inserts the other palpus. The female lies as though dead. Emerton holds that the method used in all of the spiders is for the male to discharge the seminal liquor on a little web spun for the purpose, and to dip the palpi in it; then he approaches the female and inserts the palpal organs into her epigynum. One palpal organ at a time is replaced by the other. Emerton believes that few males are eaten by females.

Montgomery has made most extensive studies of the mat ng habits of spiders (23, 24). He holds that in recognition, touch is the most important and sight is next in importance. He discredits conscious sexual selection on the part of the female.

Porter agrees with Montgomery in his statement that the female chooses that male which "first and most surely announces by his movements that he is a male." Porter found that in the *Argiope* (30) the male waits until the female has moulted, and then successfully mates with her. He found that in some cases the male left the web after approaching the female who was not ready to moult and finding her hostile. After the copulation the female wraps the male just as she does any prey, and leaves him hanging in the web with her old skin. Frequently there are as many as five male spiders on one web.

Petrunkevitch holds that sight is the only sense of sex recognition used in the hunting spiders. After sex has been recognized, courtship begins and touch is the chief means by which the male excites the female and tests her willingness to accept him (29). Petrunkevitch found that, in *Dysdera*, the male approached the female whenever she began to dig.

Nuttall and Merriam have made a study of the copulation of ticks (25). They find that there are three distinct movements in copulation. The male introduces his mouth parts into the sexual orifice of the female, dilating it and probably exciting the female; then he apposes his own sexual aperture to that of the female and expels the spermatophore, whose neck adheres to the tip of his hypostome and is then pushed into the vagina; the next movement on the part of the male is to reintroduce his mouth parts and rupture the tip of the spermatophore.

4. Conclusions.

1. In the four species of Brachyura studied, sex discrimination is tactual.

2. In the Brachyura, fertilization is effected when the female has recently moulted.

3. Males of the four species studied attempt to mate with males and fertilized females of the same species but do not attempt to mate with individuals of a different species.

4. In the Brachyura, the female is not entirely passive in the movements which precede copulation; she responds to tactual stimuli given by the male.

5. In the Crustacea, as a whole, copulation occurs at any time, regardless of the moulting of the female. It is highly probable that moulting is a necessary condition for successful fertilization.

6. In the Crustacea the female is usually passive during the act of copulation.

7. Sex discrimination in the Crustacea is kinæsthetic and tactual.

8. In the Crustacea, the males generally die soon after fertilization. A small proportion of males live for some time.

9. In the Insecta, sex discrimination is by smell; Forel's "contact-odor sense" is the fully describing term for a large degree of this discrimination.

10. Moulting has not been shown to be a necessary forerunner of fertilization in the Insecta.

11. The males of some species of Insecta die immediately after copulation.

12. In some species of the Arachnida, particularly the spiders, the females devour the males after copulation.

13. Moulting on the part of the female before fertilization is not the rule in spiders. It occurs in some genera.

14. In the ticks, the mouth parts of the male are used to moisten and stimulate the sexual aperture of the female and thus prepare the way for the injection of the spermatophore.

15. Sexual selection on the part of the female has not been definitely established in the Arthropoda. It appears that the successful male is the one who is strong enough or wary enough to mate with the female, or who has learned soonest by his senses the location of the female.

16. Since chance is a great factor in the presence of the male at the proper time, we must consider that the successful male is the one who first demonstrates his maleness to the female. Though strength is a great factor, opportuneness of proximity appears to be a greater one.

5. BIBLIOGRAPHY.

1. **Andrews, E. A.**
'95 Conjugation in an American Crayfish. Am. Nat., Vol. 29, pp. 867–873.

2. **Andrews, E. A.**
'04 Breeding Habits of Crayfish. Am. Nat., Vol. 38, pp. 165–206.

3. **Andrews, E. A.**
'10 Conjugation in the Crayfish Cambarus affinis. J. Exp. Zoöl., Vol. 9, pp. 235–264.

4. **Bell, J. C.**
'06 Reactions of the Crayfish. Harvard Ps. Studies, Vol. 2, pp. 615–644.

5. **Berlese, A.**
'01 Copulation of the House Fly. Rev. Patol. Vegetale, 9, pp. 345–356.

6. **Chidester, F. E.**
'08 Notes on the Daily Life and Food of Cambarus bartonius bartoni. Am. Nat., Vol. 42, pp. 710–716.

7. **Chidester, F. E.**
'11 The Biology of the Crayfish. Am. Nat.

8. **Darwin, C.**
'86 The Descent of Man, and Selection in Relation to Sex.

9. **Dearborn, G. N.**
'00 Notes on the Individual Psycho-physiology of the Crayfish. Am. J. Physiol., Vol. 3, pp. 404–433.

10. **Emerton, J. H.**
'78 The Structure and Habits of Spiders. Boston, B. Whidden.

11. **Feré, C.**
'98 Experiences relatives aux rapports homosexuels chez les hammetons. C. R. Soc. de Biol., T. 5, pp. 549–551.

12. **Feré, C.**
'98 Experiences relatives a l'instinct sexuel chez le bombyx du murier. C. R. Soc. de Biol., T. 5, pp. 845–847.
13. **Fielde, A. M.**
'05 The Progressive Odor of Ants. Biol. Bull., Vol. 8.
14. **Forel, A.**
'08 The Senses of Insects. Tr. by Macleod Yearsley. Methuen and Co., London, 1908.
15. **Hay, W. P.**
'05 The Life History of the Blue Crab (Callinectes sapidus). Bull. U. S. F. C., 1895, pp. 399–413.
16. **Herrick, F. H.**
'95 The American Lobster. Bull. U. S. F. C., 1895, pp. 1–252.
17. **Holmes, S. J.**
'03 Sex Recognition among Amphipods. Biol. Bull., Vol. 5, pp. 288–292.
18. **Holmes, S. J., and Homuth, E. S.**
'10 The Seat of Smell in the Crayfish. Biol. Bull., Vol. 18, pp. 155–160.
19. **Howlett.**
'07 Coupling in Empis. Entom. Mag., Vol. 43, 1907.
20. **Maeterlinck, M.**
'02 The Life of the Bee. Dodd, Mead & Co., N. Y., 1902, pp. 1–417.
21. **Mayer, A. G.**
'00 On the Mating Instinct in Moths. Ann. and Mag. Nat. Hist., Vol. 5, pp. 183–190.
22. **Mitchell, E. G.**
'07 Mosquito Life. G. P. Putnam's Sons, N. Y., 1907.
23. **Montgomery, T. H.**
'03 Studies on the Habits of Spiders, particularly those of the Mating Period. Proc. Acad. Nat. Sci. Phil., 1903, pp. 59–149.
24. **Montgomery, T. H.**
'10 The Significance of the Courtship and Secondary Sexual Characters of Aranæids. Am. Nat., Vol. 34, 1910, pp. 151–177.
25. **Nuttall, G. H., and Merriam, G.**
'11 The Process of Copulation in Ornithodorus moubata. Parasitology, Vol. 4, pp. 39–45.
26. **Pearse, A. S.**
'09 Observations on Copulation among Crawfishes with Special Reference to Sex Recognition. Am. Nat., Vol. 43, pp. 746–753.
27. **Peckham, G. W. and E. G.**
'89 Observations on Sexual Selection in Spiders of the Family Attidæ. Cramer, Aikers and Cramer, Milwaukee, Wis., 1889.
28. **Peckham, G. W. and E. G.**
'05 Social and Solitary Wasps. Houghton and Mifflin, 1905, pp. 1–311.
29. **Petrunkevitch, A.**
'10 Courtship in Dysdera croceata. Biol. Bull., Vol. 19, pp. 127–129.
30. **Porter, J. P.**
'06 The Habits, Instincts and Mental Powers of Spiders, Genera Argiope and Epeira. Am. J. of Ps., Vol. 17, pp. 306–357.
31. **Romanes, G. H.**
'83 Mental Evolution in Animals. D. Appleton and Co., 1883, pp. 1–520

32. **Stockard, C. R.**
'08 Habits, Reactions and Mating Instincts of the "Walking Stick," Aplopus Mayeri. Papers fr. Tortugas Lab., Vol. 2, pp. 43–59.
33. **Washburn, M. F.**
'08 The Animal Mind. Macmillan, N. Y., 1908.
34. **Wheeler, W. M.**
'10 Ants, their Structure, Development and Behavior. Henry Holt & Co., N. Y., 1910.